DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA MOLECULE PROGRAM ADVISORY COMMITTEE

MINUTES OF MEETING

MAY 12-13, 1975

The Recombinant DNA Molecule Program Advisory Committee was convened for its second meeting at 9 a.m., May 12, 1975 in the Pennsylvania Room, Holiday Inn of Bethesda, Bethesda, Maryland. Dr. DeWitt Stetten, Jr., Deputy Director for Science, and Dr. Leon Jacobs, Associate Director for Collaborative Research, presided for Dr. Ronald Lamont-Havers, Acting Director, National Institutes of Health. In accordance with Public Law 92-463 of January 5, 1973, the meeting was open to the public.

Committee members present were:

Dr. Edward A. Adelberg
Dr. Ernest H. Y. Chu
Dr. Roy Curtiss, III
Dr. James E. Darnell, Jr.
Dr. Stanley Falkow
Dr. Donald R. Helinski
Dr. David S. Hogness
Dr. John W. Littlefield
Dr. Jane K. Setlow
Dr. Waclaw Szybalski
Dr. Charles A. Thomas
Dr. William J. Gartland, Jr., Executive Secretary

A Committee roster is attached. (Attachment I)

The following ad hoc consultants to the Committee were present:

Dr. Elizabeth Kutter, Evergreen State College, Olympia, Washington
Dr. Wallace Rowe, NIAID, NIH

Energy Resources and Development Administration representative was:

Dr. Roy Jensen

National Academy of Sciences representative was:

Dr. Elena Nightingale

National Science Foundation representatives were:

Dr. Herman Lewis
Dr. Rose Litman

Other NIH staff attending were:

Drs. W. Emmett Barkley, NCI; Fred Bergmann, NIGMS; Earl Chamberlayne, NIAID; Irving Delappe, NIAID; Robert Martin, NIAMDD; Malcolm Martin, NIAID; Mr. Vinson Oviatt, DRS; Drs. Dilys Parry, NIGMS; Stephen Schiaffino, DRG, Gustave Silber, DRG; Bernard Talbot, OD; Katherine Wilson, DRG; Claire Winestock, DRG; Mrs. Betty Butler, NIGMS.

Members of the Press present were:

Ms. Judith Randal, New York Daily News
Ms. Cristine Russell, Washington Star
Mr. Harold Schmeck, New York Times (May 12, A.M.)
Ms. Janet Weinberg, Science News

I. CALL TO ORDER

Dr. Jacobs called the meeting to order and welcomed Committee members and guests.

II. CONSIDERATION OF MINUTES

The Minutes of the February 28, 1975 meeting were approved and accepted with the following revisions:

Page 3, line 1 is amended to read:
"Although the name of the Committee will remain as is, it will limit itself to consideration of experiments involving cell-free recombination between heterologous DNA molecules."

Page 4, top paragraph, line 7.
The sentence beginning in the middle of the line is amended to read:
"In other words, the institutional committee will evaluate the containment facilities in relation to the investigator's assessment of the risk, but not the quality and purpose of the research."

Page 4, paragraph 2, line 2:
"adequacy of the certification" is replaced by "adequacy of the assessment of the risk."

Page 5, line 1. Delete the sentence:
"The testing will probably be carried out without additional funds."

Page 5, paragraph 2, lines 3 and 8, and Page 6, paragraph 1, line 2.
"safer hosts" is replaced by "safer hosts and vectors."

Page 5, paragraph 2, line 9:
"plasmid ecology" is replaced by "E. coli and plasmid ecology."

III. REVIEW OF GRANT APPLICATIONS

After considering alternative ways of reviewing grant applications involving recombinant DNA molecules, the Committee reaffirmed its recommendation that institutional biohazards committees be involved in the review of applications in the the low and moderate risk categories. The institutional committees will certify only that the applicant has adequate facilities and adequately trained staff to comply with the NIH standards for the level of risk assessed by the applicant. The review by institutional committee will be prior to submission of the application to the NIH. It was recommended that the application form be revised so that a separate section pertaining specifically to containment facilities and procedures and the applicant's statement of assessment of risk can be the only part of the application given to the institutional committee. A certification statement from the investigator and the institution will be required. This will be in the form of a Memorandum of Understanding and Agreement. It is envisioned that the institutional biohazards committees and the Memorandum of Understanding and Agreement will also be used to handle areas of biohazards other than recombinant DNA molecules.

The NIH will issue broad guidelines on the composition of institutional biohazards committees. It was suggested that NIH might consider sponsoring workshops to train these committees. The NIH will develop a roster of consultants to assist smaller institutions and institutions with inadequate expertise with the review and certification. An alternative approach to aid the latter types of institutions would be establishment of regional review committees, although this approach will be difficult for NIH to implement.

At the next level of review the NIH Study Sections will review applications for scientific merit and the adequacy of containment. It was emphasized that containment involves both facilities and staff adequately trained in basic medical microbiological techniques. Study Sections will have the option of disapproving applications on the basis of inadequate containment for the level of risk. Study Sections will have the option of conducting site visits when judged to be necessary. It is conceivable that in certain cases, such as the high risk category, site visits may be mandatory.

Overall, the responsibility for conducting experiments involving recombinant DNA molecules lies with the applicant, the institutional biohazards review committee, the NIH Study Section, and the NIH funding institute. Dr. Bergmann indicated that he would like to refer some early moderate risk category applications to the Committee as test cases.

The Committee reaffirmed its willingness to review appeals if the need arises. Conceivably this could include appeals by NIH intramural investigators.

IV. COMPOSITION OF COMMITTEE

The Committee reaffirmed its recommendation that one layperson be appointed. For the purposes of this appointment, a layperson is defined as a non-biologist. The Committee unanimously passed a resolution calling upon the NIH to obtain nominations of people interested in the social aspects of science as possible candidates for the lay position. The Committee expressed the wish to see a list of candidates and description of their credentials.

V. BIOHAZARD CONTAINMENT FACILITIES

Dr. Barkley identified 27 biohazard containment facilities (Attachment II). An additional 9 facilities are under construction. The list does not include the NCI mobile containment facility, or ERDA facilities for the handling of radioactive materials. This list is not an endorsement of the capability of these facilities to conduct experiments safely. The facilities listed meet Center for Disease Control requirments for containment of Class 4 agents. The criteria used in the identification are as follows:

1. Sealed floors, walls and ceilings designed to prevent the penetration of microorganisms,

2. Air locks and personnel clothes change and shower areas,

3. Separate air handling systems which are designed so that air flows from areas of lowest potential hazard toward areas of highest potential hazard,

4. Provisions for filtration or incineration of exhaust air,

5. Sterilization systems for the treatment of contaminated liquid wastes, and

6. Double-door pass-through autoclaves for the sterilization of laboratory refuse.

Dr. Barkley has prepared a letter that will be sent to these facilities inquiring as to the possible availability of the facilities and the conditions which would be placed on their use.

With regard to the Frederick Cancer Center, Dr. Barkley reiterated that there is sophisticated containment space not being currently utilized, and that NCI has established a committee to determine the future need of containment space by the NCI, the NIH and other biomedical research institutions. Dr. Barkley has communicated to the NCI Committee the request of this Committee that consideration be given to maintaining portions of the available space for work involving recombinant DNA molecules. The question was raised as to whether containment equipment no longer necessary at Ft. Detrick could be used elsewhere.

The Committee still does not consider the construction of high containment facilities to be of high priority.

VI. CONSTRUCTION AND TESTING OF SAFER HOSTS AND VECTORS

A draft announcement soliciting the construction and testing of safer bacterial hosts and vectors, prepared by a subcommittee composed of Drs. Curtiss, Falkow, Helinski and Szybalski, was reviewed. The final version appears in Attachment III. It was felt that this solicitation should not be so specific so as to preclude novel approaches. Unless it is decided that protocols can and should be specified, it is anticipated that the grant mechanism can be used for construction of safer hosts and vectors. There was concern, however, that these grant applications would not fare well in Study Section scientific merit reviews. It was felt that these applications would have to be segregated from research project applications for review and funding purposes. During this discussion it was brought out that there are short and long range aspects to the development of safer hosts and vectors, and some aspects may be more appropriate for support by grants, and others by contracts.

The question was raised as to whether the Committee should make recommendations to investigators on which host-vector systems are safer now. It was felt that the Committee has no mechanism to make such a judgment now, and perhaps will never be in a position to advise specifically on the use of preferred strains. However, the assignment of a risk level to an experiment will take into account the host-vector system to be used.

The Committee felt that the word "safer" should be defined in a statistical sense. It was emphasized that the host and cloning vehicles should be considered as a composit system. For example, the unit number 1 could be assigned to a given host-vector combination, such as wild type E. coli K-12 and the ColE1 plasmid. A host-vector combination would be defined as safer than the unit standard if its survival, or other property, were reduced by a certain number of logs. A given host-vector system would then be tested to see if it meets the decided-upon requirements.

The Committee felt that it is a matter of high priority to establish a program for testing safer hosts and vectors. It is desirable that the testing be carried out independently of the construction. The testing program will most likely be carried out under contract. It is anticipated that Requests for Proposals for testing will require the tester to accept the Committee's definition of the unit standard, and will specify the precision of testing required. The Committee will provide advice on the development of protocols for in vivo and in vitro testing. The testing program will have to take into consideration the consequences of ingestion of the strain by laboratory workers, and of escape of the strain from the laboratory.

The Committee felt that some simple first phase testing, such as survival of hosts and vectors, should begin as soon as possible. Because of the urgency for a testing program and the delays inherent in requesting and reviewing contract proposals, the Committee asked Dr. Jacobs to determine whether NIH could begin a testing program using facilities at the Frederick Cancer Research Center while a contract program is being developed.

VII. GUIDELINES FOR RESEARCH

The Committee recommended that the final Asilomar Conference report be used as a provisional guide for research involving recombinant DNA molecules until NIH guidelines are adopted. A subcommittee of Drs. Hogness (Chairman), Chu, Helinski and Szybalski plus ad hoc consultants will work on drafting NIH guidelines for research, and will report to the full Committee in July. The Committee recommended that the subcommittee define 5 classes of experiments taking into consideration risk, physical containment and biological containment. The subcommittee will try to incorporate into its guidelines a downgrading scale based on test data. The question was raised as to whether downgrading should be done on the basis of laboratory data, or whether it must await the results of animal and human testing. The latter course will result in considerable delay of experiments. The question was raised as to whether risk can be factored into two components: the likelihood of escape and the effects of such an escape. Little information is available on the latter, however.

VIII. STOCK CENTERS

Dr. Adelberg reported that the stock center at Yale will be able to store and distribute safer strains of E. coli as they become available. Storage of species other than E. coli in this collection is uncertain. The Yale collection will also handle strains carrying plasmids until a plasmid collection is established. Dr. Szybalski's laboratory will distribute safer phage until a phage center can be established. Dr. Jacobs agreed to investigate the capabilities of other laboratories and institutions for the maintenance and distribution of stocks.

IX. NEWSLETTER

The Committee discussed the NIAID proposal for a newsletter in its Scientific Memoranda series. It recommended that this Memoranda series be restricted solely to in vitro synthesized recombinant nucleic acid molecules. NIAID agreed to establish the Memoranda series in this format. The revised project description appears in Attachment IV. The Scientific Memoranda are informal communications, and can not be referenced. They are distributed only to active investigators who provide at least one contribution a year, although there is a 2-year grace period at the beginning. This memoranda series will

be started on a quarterly basis, and the initial mailing will be to Asilomar attendees, and perhaps a subset of the NIH Guide for Grants and Contracts distribution list.

There was concern that a newsletter in this format would not provide widespread distribution of information on strains, guidelines, safety, equipment, etc., to investigators considering entering the field. Announcements placed in this Memoranda series would also have to be placed elsewhere so that non-members of the contributing group would have access to the information. Perhaps subsections of the Memoranda could be sent to a larger audience. Announcements in Science, Nature, the Proceedings of the National Academy of Sciences, and the NIH Guide for Grants and Contracts will most likely be necessary.

X. ECOLOGY OF ESCHERICHIA COLI

Dr. Falkow summarized his report on the ecology of E. coli (Attachment V). He will continue to update this information. This sort of information should also be gathered for phage.

It was recommended that anyone doing experiments with recombinant DNA molecules involving E. coli K-12 in any risk category should include a marker which will unequivocally permit identification of the organism. Nalidixic acid resistance or arabinose and mannitol minus markers could be used. The question was raised as to whether NIH guidelines should require monitoring of laboratories engaged in moderate and high risk experiments. The suggestion was made that perhaps as a first step there should be a contract to do a monitoring exercise of some standard laboratories to obtain data on how well they are contained. If monitoring is adopted as a policy it should probably be carried out under the auspices of an institutional biohazards safety committee, which would be independent of the laboratory and could have policing authority.

XI. ASSESSMENT OF HAZARDS

The question was raised as to what information will be needed to move an experiment from high to moderate risk containment. The question of whether foreign DNA can change the pathogenicity of E. coli in unconceived ways was also raised. Perhaps studies should be supported by grants or contracts to try to answer some of these questions.

XII. TOUR OF BUILDING 41

Dr. Barkley conducted a tour of Building 41, a containment facility of the National Cancer Institute.

XIII. NATIONAL SCIENCE FOUNDATION PROCEDURES

Dr. Lewis described procedures being used by the NSF for handling applications involving recombinant DNA molecules. The NSF is requesting a letter of understanding from investigators indicating that they are aware of the Asilomar principles, and agree to follow good laboratory practices. Investigators also agree not to alter protocols without prior NSF approval. These are viewed as interim procedures until institutional biohazards committees are established and functioning.

XIV. WORKSHOPS

The Committee recommended that the NIH should develop plans for training in the area of basic medical microbiological techniques. Options could include regional courses, workshops, meetings, audio-visual aids, etc.. Regional courses involving the demonstration of equipment and techniques would be valuable, but are difficult to conduct.

The Committee recommended that a workshop be held on the development and testing of safer bacterial hosts and vectors. A subcommittee of Drs. Helinski (Chairman), Curtiss and Falkow will be responsible for organizing this workshop, and submitting a proposal to the NIH. It is hoped that the workshop can be held in October or November. It was suggested that a subsequent meeting of the Committee could be held in conjunction with this workshop.

Dr. Kutter will investigate the possibility of having an informal session on safer phage at the phage meeting at Cold Spring Harbor in August.

A subcommittee of Drs. Darnell (Chairman) and Malcolm Martin, with the assistance of Dr. Sambrook, was appointed to plan a separate animal virus workshop, possibly in conjunction with a meeting at Cold Spring Harbor.

XV. NEXT MEETINGS

The next meeting of the Committee will be on Friday and Saturday, July 18 and 19, 1975. The following meeting may be held in October in conjunction with the workshop on construction and testing of safer bacterial hosts and vectors.

XVI. ADJOURNMENT

The meeting was adjourned at 4 p.m. on May 13, 1975.

I hereby certify that, to the best of my knowledge, the foregoing minutes and attachments are accurate and complete.

June 6, 1975
Date

DeWitt Stetten, Jr., M.D.
Acting Chairman, Recombinant DNA Molecule Program Advisory Committee
National Institutes of Health

William J. Gartland, Ph.D.
Executive Secretary

RECOMBINANT DNA MOLECULE PROGRAM ADVISORY COMMITTEE

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BIOHAZARD CONTAINMENT FACILITIES

Facilities	Approximate Area Square Feet	Director (address)
Abbott Laboratories	12,000	Mr. Ted Leonards, President 1400 Sheridan Road North Chicago, Illinois 60064
California, University of-Davis	1,000	Dr. D. Duntworth, Director Davis, California 95616
Center for Disease Control	4,000	Dr. Sencer, Director 1600 Clifton Road, N.E. Atlanta, Georgia 30322
Columbia, University of	500	Dr. Sol Spiegelman, Director Institute of Cancer Research 99 Fort Washington Avenue New York, N.Y. 10031
Delta Regional Primate Research Center	1,350	Dr. Peter Gerone, Director Tulane University Covington, Louisiana 70433
Duke University	7,200	Dr. Daryl Bigner, Director Associate Professor of Pathology P.O. Box 3156 Durham, North Carolina 27710
Electro-Nucleonics Laboratories, Inc.	10,000	Dr. Charles Neilson, Chief Executive Officer 12050 Tech Road Montgomery Industrial Park Silver Spring, MD. 20904

Facilities	Approximate Area Square Feet	Director (address)
Frederick Cancer Research Center	330,000	Dr. William Payne, Scientific Coordinator Building 550, Ft. Detrick Frederick, Maryland 21701
IIT Research Institute	12,000	Dr. Richard Erlich, Director 10 West 35th Street Chicago, Illinois 60616
Lyndon B. Johnson Space Center Lunar Receiving Laboratories	11,000	Dr. Christopher C. Craft, Director Houston, Texas
Dugway Biocontainment Facility	15,000	Dr. Paul Adams, Chief Life Sciences U.S. Army Dugway Proving Grounds Dugway, Utah 04022
Life Sciences, Inc.	16,500	Dr. Vincent Groupe, Director 2900 72nd Street, North St. Petersburg, Florida 33710
Merck and Company, Inc.	10,000	Dr. M. Hilleman, Director West Point, Pennsylvania 19485
Merill National Company (Div. of Merill Richardson)	8,000	Dr. John S. Lawlis, Vice-President Biological Operations Swiftwater, Pennsylvania 18370
Meloy Laboratories, Inc.	10,000	Dr. Verna, Director 6715 Electronic Drive Springfield, Virginia 22151
National Animal Disease Center	34,000	Dr. P. A. O'Berry, Director P.O. Box 70 Ames, Iowa

Facilities	Approximate Area Square Feet	Director (address)
NCI, Building 41	15,000	Dr. J. B. Moloney, Assoc. Director Viral Oncology Building 37, Room 1A-13 Bethesda, Maryland 20014
National Center for Toxicological Research	3,000	Dr. Morris Cranmer, Director Jefferson, Arkansas 72079
NINCDS, Building 36	8,200	Dr. J. Severs, Director and Chief Infectious Disease National Institutes of Health Bethesda, Maryland 20014
Naval Biomedical Research Laboratory	20,000	Dr. Neyland Vedros, Director Naval Supply Center Oakland, California 94625
Oak Ridge National Laboratory	4,000	Dr. Herman Posta, Director Oak Ridge, Tennessee
Ohio State Research Foundation	8,000	Dr. David Yohn 1314 Kinnear Road Columbus, Ohio 43212
Plum Island Animal Disease Center	22,000	Dr. J. Callis, Director Dr. Greeves, Associate Director P. O. Box 848 Greenport, New York 01944
St. Louis University	2,500	Dr. M. Green, Director and Professor Institute of Virology 3681 Park Avenue St. Louis, Missouri 63110

Facilities	Approximate Area Square Feet	Director (address)
U.S. Army Medical Research Institute of Infectious Diseases	60,000	Col. Joseph Metzger Ft. Detrick Frederick, Maryland 21701
New York State Department of Health	?	Dr. Donald Dean, Director Division of Laboratories and Research New Scotland Avenue Albany, New York 12201
University of Kentucky at Lexington	10,000	Dr. Goodman, Head Mycology Isolation Unit Lexington, Kentucky 40506

Contract proposals for the construction and testing of safer cloning vehicles and hosts for research involving recombinant DNA molecules are solicited by the National Institutes of Health.

Recently developed techniques that permit the combination of genetic information from heterologous organisms have focused attention upon the need for DNA cloning vehicles (plasmids, bacteriophage) and bacterial hosts with a restricted capacity to multiply outside the laboratory. The basic aim is to reduce the potential biohazards of cloned recombinant DNA molecules by a significant factor.

Proposals specifically should pertain to: (1) improving the safety and efficacy of existing cloning vehicles and host systems, and (2) the development of new cloning vehicles and host systems.

A. Construction of a Safer Phage Cloning Vehicle and Bacterial Host System—

Principle: A safer phage cloning vehicle should be able to propagate only in a unique laboratory adapted host, and be unable to establish a prophage or carrier (plasmid) state. Thus, its dissemination in nature would be self-limiting.

In addition, combinations of the following features would further increase the safety and/or utility of the system:

(1) Ability to produce very high phage yields (this reduces the volume of the culture and concomitant risks);

(2) Introduction of stable and well characterized mutations that would render phage particles inactive or very unstable in nature;

(3) Inability to transfer the cloned DNA to bacteria common in nature;

(4) Capacity to integrate both small and very large fragments of DNA;

(5) Ease of construction and detection of DNA recombinants;

(6) Well characterized genome of the phage and the host.

B. Construction of a Safer Plasmid Cloning Vehicle and Bacterial Host System--

Principle: A safer plasmid cloning vehicle should only replicate in a unique laboratory adapted host and be non-self-transmissible and poorly or non-mobilizable. The host bacterium should be dependent for growth on unique laboratory controlled conditions and be unable to transfer the resident plasmid to another bacterium.

In addition, combinations of the following features would further increase the safety and/or utility of the system:

(1) Defective replication of the cloning vehicle and/or killing of the host bacterium at mammalian body temperature;

(2) Destruction of the plasmid and/or host chromosomal DNA at mammalian body temperature;

(3) Inability of the host to integrate the plasmid DNA into its chromosome;

(4) Inability of the host to be infected by potential transducing bacteriophage.

C. Testing of Safer Cloning Vechicles and Hosts--

Principle: Monitoring procedures should be developed to detect the hosts and safer cloning vehicles in order to measure the survival and growth of the host and transmissibility of the vehicle in natural environments.

Specifically, in comparison to the wild-type cloning vehicles and hosts, the safer cloning vehicles and host cells should be tested with regard to:

(1) Survival in man or other appropriate mammalian hosts;

(2) Survival in the laboratory environment during normal experimental manipulations;

(3) Survival on plants, in soil and in aquatic environments;

(4) Transmissibility of safer cloning vehicles from safer hosts in natural environments.

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014, U.S.A.

PROJECT DESCRIPTION

"Recombinant Nucleic Acid Scientific Memoranda" (RNSM)

Background: This project is part of a feasibility study, being conducted by the National Institute of Allergy and Infectious Diseases, NIH, USPHS, to develop a new means (Scientific Memoranda) for rapid and informal scientific communications between active investigators in narrow and selected fields of research. It is not the purpose of the project to provide information services to others.

To be included in this study, the group of investigators involved must be relatively small in number, scattered geographically, conducting investigations on the leading edge of knowledge, with research that is progressing rapidly or in need of stimulation. The projects are evaluated annually and an Institute decision made on continuation of each of them. Changes in the frequency, format, etc. of the "Scientific Memoranda" are made as experience indicates.

Origin of the RNSM: The International Conference on Recombinant DNA Molecules took place at the Asilomar Conference Center, Pacific Grove, California during 24-27 February 1975. This Conference was called by the National Academy of Sciences and funded by the National Institutes of Health and the National Science Foundation. The Conference culminated a series of discussions and meetings held previously on the potential biohazards associated with experimentation involving recombinant DNA molecules. In the consensus report presented at the end of the "Asilomar Conference" by the organizing committee, one of the recommendations called for the establishment of a fast and informal communication system.

The National Institute of Allergy and Infectious Diseases concurred with this recommendation and responed promptly by establishing the RNSM for communications concerning research on recombinant nucleic acid molecules constructed in vitro, including:

(a) Development of methods for the construction of such molecules, including the preparation and characterization of appropriate enzymes;

(b) Study of the expression of such molecules in different host cells;

(c) Development and use of effective and relatively safe cloning vehicles and host cells; and

(d) Determination and reduction of their potential hazard.

What is the RNSM? It is a collection of photocopies of brief and informal scientific communications contributed by the investigators participating in the project. All accepted contributions contain opinions, suggestions, or tentative data that are subject to revision before formal publication. Appearance of information in the RNSM does not constitute publication. It is solely for the personal use of the participating investigators and should be treated as "Personal Communications."

Contributions are composed of brief descriptions of ongoing research projects, progress notes, abstracts and summaries of unpublished findings, along with tables, figures and photographs to clarify the text. Negative data are important contributions. Informal reports on scientific meetings, technical suggestions, discussion and criticism, and notes on bibliographic omissions, as well as any other informal communications on current research work are acceptable. Annexed to each distribution of the RNSM is a list of "Bibliographic Notices" contributed by the participants. This is a listing (title, authors and journal) of all formal manuscripts, involving research relevant to the areas outlined above, as they are published.

Contributions to the RNSM are not edited but are monitored by the Project Officer to assure that they do not mimic or replace formal manuscripts that belong in the scientific journals. Contributions to the RNSM cannot contain sections on Introduction, Materials and Methods, References, or Acknowledgments. They must be short and informal in nature.

Participants: Any bona fide investigator, in any part of the world, conducting research in the areas outlined above, and who agrees to abide by the conditions of this project. Evidence of the active research endeavor must be presented in the form of a minimum of one scientific contribution to the RNSM per year. More frequent contributions are preferred. As this is a scientist-to-scientist communication project, investigators are encouraged to participate on an individual basis, rather than group participation, although multi-author communications are acceptable.

A scientist may be nominated for participation in the RNSM by a colleague, he may nominate himself, or be nominated by the Project Officer. Each nominee receives a standard invitation letter, in duplicate, which describes the project and the conditions of participation. When an individual countersigns his willingness to participate and abide by the conditions, and returns one signed copy

of the letter, his name is placed on the mailing list and remains there until he withdraws it, or one year after his last contribution to the RNSM. Investigators must contribute communications to receive the RNSM. Otherwise, no fee is charged for this service.

Periodically, a list of current participants with their mailing addresses is annexed to a distribution of RNSM. This provides contact points for the participating investigators.

Distribution of the RNSM: This occurs quarterly, with the closing date being the 15th of March, June, September and December. Following each closing date the accumulated contributions are collated, photo-reduced, and reproduced. Distribution is via airmail, except to addressees east of the Mississippi in the U.S.A.

Submission of Contributions to the RNSM Contributions must be typed on plain 8" x 10½" paper and on one side only. Single spacing should be used and all pages including table and figures should be numbered. Original and one copy of each contribuion are sent to: Dr. EARL C. CHAMBERLAYNE; PROJECT OFFICER, NIAID: BLDG. 31, RM 7A50; NATIONAL INSTITUTES OF HEALTH: BETHESDA, MARYLAND 20014, U.S.A.

To: Members
Recombinant DNA Molecule Program Advisory Committee, NIH

From: Stanley Falkow

RE: THE ECOLOGY OF ESCHERICHIA DNA

The Definition of the 'Species' Escherichia coli

Escherichia coli (Castellani and Chambers) is the type species of the genes Escherichia- a member of the family Enterobacteriaceae. Cells of this species are nonphotosynthetic, non-sporulating gram negative rods. They are either motile with peritrichous flagella or nonmotile. They are heterotrophs and facultative anaerobes. The vast majority of strains isolated from nature are prototrophic. The genus Escherichia is differentiated from other genera by the constellation of characteristics listed in appendex 1. The principal differentiating biochemical characteristics of E. coli that are employed for differentiation from other Enterobacteriaceae are: a) indole production b) failure to utilize citrate as a sole carbon source c) the ability to produce gas from glucose and d) the ability to ferment lactose. None of these characteristics per se are particularly useful as a distinctive identifying marker for a host strain of E. coli to be used for DNA recombinant molecule experiments. My initial assessment of the common biochemical reactions exhibited by most isolates of E. coli indicates that the use of an arabinose non-fermenting (ara^-) and/or mannitol- nonfermenting (mlt^-) strain would be advantageous. The ability to ferment these substrates is common to virtually all lactose-fermenting species of enteric bacteria so that a mtl^-/ara^- negative lactose fermenting host for DNA recombinant molecule experiments would constitute a reasonably distinctive pattern useful for monitoring. The use of a lac^+ ara^-/mtl^- host cell together with a distinctive marker on the cloning vehicle could likely be used in combination to provide effective general monitoring of dissemination by laboratory procedures and even for fecal monitoring. Obviously, as new host strains are generated over the next few years better markers will surely become available. However, for the immediate problem at hand, these markers seem potentially useful. All in all, however, the common biochemical tests applied to the characterization of E. coli are not sufficient to provide a sound basis for differentiating

between strains for epidemiological and ecological purposes.

Serology has provided the most satisfactory method for epidemiological and ecological studies. Serological classification is made on the basis of typing of somatic (O), capsular (K) and flagellar (H) antigens. There are currently 150 recognized O antigens whose specificity is based on the arrangement and composition of polysaccharide moity of cell wall lipopolysaccharide. The capsular (K) antigens are subdivided into three groups A, B and L. A antigens are true capsules, while B and L antigens are generally formed as "microcapsules" except when the strains are grown at 15-20°. In all, some 99 distinct K antigens are recognized. Most K antigens are lipopolysaccharide in nature, although recently several K antigens have been recognized as proteinaceous appendages of the cell. These latter K antigens are of great epidemiological significance. Three of these antigens K88, K99 and the provisional K100 are absolutely essential to the strain to ensure colonization of the porcine, bovine and human small bowel, respectively. While these K antigens are generally found on enteropathogenic E. coli there is other data accumulating to indicate that the success of colonization of the bowel, colonization of the bladder and even the specificity of colonization of certain plants are largely determined by specific K antigens. The flagella of E.coli are also antigenically specific. Some 51 distinctive heat-labile protein antigens (H antigens) are recognized.

The utility of serogrouping of E. coli for epidemiological studies has been primarily shown in tracing E. coli strains which are etiological agents in infantile diarrhea. The significance of O serogrouping in broader epidemiological and ecological investigations has been limited. The data which are available show that O serotyping may be useful, although obviously when one is dealing with 150 distinct types investigations become somewhat laborious and, of necessity, limited in scope. Of course, once one can establish the prevalence of a particular serotype with a particular disease state, ecological niche etc. it can be exploited for wide scale application. Unfortunately,

such instances are relatively rare and O serotyping is generally of most use in studying small relatively isolated populations. Even when studying relatively limited populations, however, the task often becomes complex. For example, F. J. Skerman and I examined the prevalence of E. coli serotypes in dairy herds and the waterways in the northern sector of the Brisbane Valley between November 1968 and May 1969. Among the 242 isolates examined from all sources, 76 of the 150 known O antigens were identified, although three O groups, 08, 088 and 025 predominated. Among the members of a single dairy herd, we found 37 of the 150 known antigens of which the three O groups 018, 068 and 0150 predominated. This 'distinctive' herd pattern permitted us to gain some idea of the contribution of the herd to the fecal pollution of nearby waterways and the survival of these E. coli. There has recently been a good deal of effort in England to correllate the presence of R plasmids with specific O serotypes of E. coli. This approach has proved useful since it appears that fully 25% of R^+ strains are found in only 3 O serogroups. A number of individuals are also attempting to use O serogrouping to assess the extent to which E. coli of animal origin colonize humans. It is not yet clear if there are exclusively 'animal' and 'human' enteric bacteria let alone if the two floras overlap.

H antigen typing has only been used as an adjunct to O serogrouping and has not proved significant alone. K serotyping seems of great epidemiological significance. It is currently employed primarily as an adjunct to O serogrouping. K typing is considerably more difficult, however, since the K antigens are rather rapidly lost in the laboratory. From the basic scientific standpoint, it would be obviously useful if there were a more widespread application of serotyping to study the ecology of E. coli. It is, however, a complicated, expensive and often unrewarding task. From the specific standpoint of the detection of E. coli host strains used for recombinant DNA molecules and their dissemination serotyping appears at present to be of no significant value.

E. coli K-12 is semi-rough and cannot be assigned to any of the 150 known O serogroups. It does not possess A antigen and its H antigen is not distinctive. One may wish to consider making a 'safe' host that has a unique serotype, although the long term utility of the approach seems somewhat doubtful to me.

Phage sensitivity patterns similar to those used for typing S. typhimurium have not been widely applied to strains of E. coli in ecological and epidemiological studies. Nor is their a recognized 'panel' of phages available for use.

Gastrointestinal flora and the role of E. coli

The natural habitat of E. coli is the alimentary tract of man and warm blooded animals. E. coli strains form a relatively minor proportion of the intestinal microflora in numerical terms (generally < 1%). Apart from a significant effect of breast feeding, normal nutritional changes do not drastically change the composition of the gastrointestinal flora. Experimental feeding with a prevailing meat-egg, milk vegetable or vegetable diet does not lead to dramatic differences. Increased consumption of lactose (40g/day) or yogurt has no effect nor does an increased diet of cellulose. (The possible effects of an E. coli possessing a DNA recombinant molecule conferring cellulase activity has been often used as an example of a potentially deleterious organism. These basic observations on the failure of diet to change the microflora may either mitigate or intensify such fears depending upon one's attitude).

It has been suggested that colicins produced by many E. coli (up to 42% in some surveys) could interfere with the multiplication of other organisms of the bowel and provide a selective advantage. Most studies have demonstrated, however, that colicins are not usually produced in the bowel. On the other hand, antibiotics used in therapuetic and subtherapuetic doses may have a profound effect on the normal flora, rendering the animal susceptible to infection by pathogens and enhancing to plasmid transfer (see below).

The E. coli composition of the intestinal flora is complex. If one examines the feces of any one individual over a period of time serotyping generally reveals a continuous succession of different antigenic E. coli strains. In one study, for example, the number of antigenic types present in each of six individuals varied from 2 to 9 at any time over a six month period. In another study of 4 individuals over a period of two and one-half years, it was observed that the E. coli flora at any particular time consists of strains which persist for relatively long periods of time (resident strains) accompanied by three or four strains which persist for only a few days (transient strains). Resident strains persist for several months but eventually are replaced by other strains. The change in a resident strain(s) in any individual is not necessarily accompanied by any manifest symptoms such as diarrhea. Rather, the establishment of a new resident strain appears to be a gradual process. The factors which influence the change of resident is not known. Attempts to replace the resident strain by ingestion of known E. coli types is rarely successful (see also below).

Escherichia do not normally colonize the stomach and proximal small bowel of adults in significant numbers. Indeed, abnormality of the gastrointestinal tract is often recognized by the appearance of E. coli and other coliform organisms in these regions. Babies may periodically show a dysbiotic flora following minor infections, vaccinations or sudden changes in diet, although in normal babies the condition is transient and disappears rapidly. The causes of dysbiosis in adults have been associated with anacidity, disturbance of peristalsis, surgery of the gastrointestinal tract, diseases of liver or kidney, pernicious anemia, the blind-loop syndrome and antibiotic therapy. A dysbiotic flora is not uncommon with advancing age. All of these parameters were initially considered in the report of the Plasmid Group to the Asilomar Conference and led to the recommendations that individuals should avoid working with E. coli containing recombinant DNA molecules if they

were receiving antibiotics, had functional gastrointestinal disorders or had experienced extensive bowel surgery or were ingesting large amounts of antacids Other factors concerning the colonization of individuals with E.coli will be discussed when describing the in vivo transmission of plasmids in a later section.

Role of E. coli as a Pathogen

The role of E. coli as a pathogen has been well documented. Specific serotypes of E. coli have been associated with human neonatal and infantile diarrhea particularly 026:B6, 055B5; 086:B7, 0119:B14, 0124:B17, 0125:B15, 0.26:B16, 0127:B8 and 0128:B12. The gastroenteritis caused by these enteropathogenic serotypes resembles that seen in salmonellosis. Other E. coli serotypes, specifically strains with the O antigens 028ac, 0112ac, 0124, 0136, 0143 and 0144 have been implicated in disease of children and adults which resembles bacillary dysentery in that the inflammation of the colonic mucosa gives rise to ulcerative lesions resulting in blood, epithelial tissue and mucous in the stool. The serotypes which give rise to the Shigella-like syndrome differ from those giving rise to the Salmonella-like syndrome in that they cause keratoconjunctivitis in the rabbit eye (Sereny test) which is a test correllated with active lethal penetration of epithelial cells.

In recent years by far the most active research on the etiology of E. coli diarrhea has centered around strains known to produce enterotoxins. These toxigenic strains cause sporadic "traveller's diarrhea" in adults and are endemic in many developing countries in members of all age groups. The E. coli enterotoxins are plasmid-mediated and toxigenic strains also generally contain a plasmid specifying a specific K antigen which permits the host organism to specifically adhere to the small bowel mucosa which greatly favors successful colonization of an animal. Most cases of neonatal diarrhea in calves and piglets are caused by toxigenic E. coli. Serotyping is of somewhat limited

value in many instances since virtually any serotype may harbor an Ent$^+$ and K plasmids. In a practical vein, however, if one deals with isolated populations of animals or man one often finds that all toxigenic strains over a particular time frame belong to but 1 or 2 O serotypes.

By far and away, the most common cause of urinary tract infection in man is E. coli. These E. coli generally enter the bladder from the patients own fecal flora by an ascending route. The epidemiology of urinary tract infection is still rather obscure, although as noted earlier certain strains which possess particular K antigens may be extremely important. Among hospitalized patients E. coli is a leading cause of septicemia. In recent years, it has been assumed that this represents opportunistic infection in debilitated patients or those compromised by immunosuppressive therapy. It has been recently shown, however, that E. coli which possess the ColV plasmid are far more likely to be isolated from extra-intestinal infections. In short, E. coli is not ordinarily a highly virulent organism. Certain organisms have the inherent potential for invasiveness or may acquire sufficient accessory genetic information, usually plasmid-mediated, that is sufficient to tip the balance from a strain that is usually a commensal to one which is capable of initiating overt disease. From the standpoint of recombinant DNA molecules, the documentation of the effects of plasmid-mediated determinants on pathogenicity must be viewed as one of the most cogent arguments for the potential biohazards associated with this research.

Occurrence, dissemination and survival of E. coli

Although the usual habitat of E. coli strains has been well established as the alimentary tract of man and warm-blooded animals they are found widely distributed in nature. They are widespread in soils from both densely inhabited and sparsely inhabited regions. E. coli have also been isolated from insects of the orders Coleoptera (beetles), Orthoptera (grasshoppers), Hymenoptera

(leafhoppers), Lepidoptera (butterflies and moths) and, of course, the Diptera (flies) and it is suggested that these insect vectors may be responsible for the wide-spread dissemination of E. coli strains. Birds and fish which have been in proximity with man apparently disseminate E. coli and other coliform organisms. Thus, it would appear that there are many vectors participating or capable of participating in the dissemination of E. coli. In the "worst case" analysis of the dissemination of an E. coli carrying a 'hazardous' DNA recombinant molecule one would be faced with an enormous number of potential vectors for dissemination. The numbers of E. coli disseminated by these 'unusual' vectors are significant. Although only small numbers of E. coli have been isolated from insects, it has been estimated that a gull disseminates 50 to 100 x 10^6 E. coli per day (I cannot help but be reminded of a typhoid epidemic traced back to gulls feeding upon sewage). Obviously, many wild animals disseminate large numbers of E. coli.

Changes in the E. coli flora must occur through their ingestion. Exchange of strains between members of the human population occurs primarily via personal contact. It is not certain, however, to what extent man acquires his E. coli from domestic animals (and vice versa) or from plants. The role of E. coli from animal sources as a potential colonizers of man and as potential sources of human infection has been argued to be of dubious significance. Yet, recent evidence indicates that the predominant 08, 09 and 0101 antibiotic resistant serotypes found in human feces is likewise found in antibiotic resistant E. coli of calves. The possibility of significant exchange of E. coli between man and animals by contaminated food products cannot be ruled out. E. coli has been found widespread on plants so that exchange of E. coli between animals may not only occur by direct contact but also by ingestion of contaminated grass and water.

Possibly the major question with respect to the dissemination of E. coli revolves about its ability to survive in soils and water. It is known that E. coli

continues to multiply for a period of time in freshly voided feces. All of the information available indicates that in warm, moist soil conditions E. coli remain viable for a considerable period of time. As but one example, a soil area ungrazed for 3 years yielded 25 x 10^5 E. coli per 100g. Obviously, the survival in soil is quite dependent upon pH as well as moisture. E. coli dies more rapidly in acidic organic soils than in limestone soils. Organisms from vegetation and surface contamination of soils may be ingested by animals but are also washed into adjacent waterways. Since soils act as reasonably effective filters subterranean drainage to water courses may occur but is limited to areas very closely adjacent to watersources. The most efficient contamination of watercourses is by surface drainage during heavy rainfalls or by direct contamination such as occurs in (too) many large urban areas in the U.S. In the latter context, during our examination of fecal pollution of the Potomac River and Rock Creek in the Maryland-D.C.-Virginia area in 1969-1971 it was common to find the fecal E. coli increase from 10^3 to 10^8 per 100 ml following a heavy rainfall.

The survival of E. coli strains in water is of considerable importance for the dissemination of these organisms. There are numerous reports that E. coli survive for a long period of time in fresh water. For example, E. coli survive for 9 months in water inoculated with feces. The major factors which affect the survival of E. coli cells in natural watercourses are a) the flow rate b) level of inorganic salts c) sunlight and d) predation (particularly by protozoa and myxobacteria). Under certain conditions E. coli cells multiply in water. This is particularly true of heavily polluted water containing organic compounds. In fact, the organisms may multiply to a level equivalent to that of a laboratory broth culture. E. coli do not fare so well in tidal estuaries since the organisms die rapidly. Thus, 99% of E. coli are destroyed in a tidal estuary by a combination of dilution, predation and particularly the bactericidal action of sea water (principally due to organic salts). However, if sufficient organic

matter is present, E. coli cells can survive and even multiply in tidal estuaries despite the generally unfavorable conditions.

The Plasmids and Bacteriophages of E. coli

As was indicated in the report of the Plasmid group to the Asilomar Conference, plasmids and bacteriophages are very common in E. coli. I have not been able to find very much additional useful information concerning phage ecology other than that given to the Asilomar Meeting. In the following Table I have given some data we collected from a survey of 260 E. coli from the upper Brisbane Valley in Australia. These data were collected to serve as base line figures since the Valley was isolated and the human and domestic animal residents were not regularly seen by physicians or veterinarians and antibiotics were not employed in animal rearing.

Plasmid Incidence in Wildtype E. coli Strains from Human, Bovine and Equine Sources

Source	Total No. of Strains	No. Carrying Conjugative Plasmids	Plasmid Type				
			F	I	Untypable fi^-	No Col^+	Hly^+
Human	43	12(28%)	3	3	6	6	0
Bovine	134	58(44%)	34	4	20	8	4
Equine	52	17(33%)	10	2	2	3	0

These data simply show the incidence of self-transmissible plasmids in these E. coli strains which were all, incidentally, antibiotic sensitive. If one considers that many of these isolates also harbored non-transmissible plasmids and that our screening procedures would not be considered to be better than 85% efficient, the very large pool of plasmids species in E. coli become apparent. These data are not unique since H. Williams Smith and M. Richmond (personal communications) have observed similar results. Indeed, their results showed an even higher carriage of transmissible plasmids since many of their isolates contained R-factors. The R plasmids do, in fact, place an increased plasmid burden upon E. coli in

modern society. For example, fully 2% of all coliforms obtained in the sewage outflow from domestic housing in Bristol, England carry R plasmids. Perhaps the most startling conclusion of the Bristol study was that more than 85% of all R plasmids are carried by bacteria inhabiting the population outside hospitals. This observation seems to stress a vital point in the ecology of R plasmids: any R plasmid transfer or colonization of people with R^+ bacteria that occurs is most likely to take place in people who are not undergoing purposive antibiotic treatment. It would be possible to go on for many pages concerning the ecology of plasmids in E. coli. However, it is sufficient, I think, to simply conclude that plasmids are present in a signficant proportion of E. coli and in the context of recombinant DNA molecules, the 'indigenous' plasmid flora of E. coli would represent (at least in theory) a ready body of vehicles to mobilize and recombine with the laboratory constructed molecules under the proper circumstances. However, the critical point is to what extent plasmid transfer occurs in vivo.

Plasmid Transfer in vivo:

As you know E. S. Anderson, H. Williams Smith and Mark Richmond all presented data at the Asilomar Conference on the survival and/or the plasmid transfer of E. coli K-12 in the human bowel. Drs. Anderson and Smith were kind enough to send me preprints of their work prior to publication and these are found in Appendix II. Both studies are in substantial agreement that E. coli K-12, which is at present the favored host organism for DNA recombinant molecules, is a poor colonizer of the human alimentary tract, although Anderson presents evidence that E. coli K-12 multiplied to some extent in 2 of 8 subjects. Smith found no transfer of an R-factor from K-12 to resident enteric flora whereas Anderson was successful in detecting plasmid transmission if the E. coli were fed in substantially high numbers. However, the cells receiving the R plasmid did not presist nor did they apparently, in turn, transmit the plasmid to other E. coli. Studies in my own laboratory are essentially the same as those reported by Anderson and Smith. Moreover, we

have observed similar findings in calves. We have also detected transfer of carried R plasmids in vivo, although as noted by Anderson, the converted strains do not persist in the absence of antibiotic selection. One interesting facet of our work was that non-plasmid containing E. coli K-12 fed to calves while surviving for only 3 days, nevertheless, acquired plasmids from the indigenous flora. The E. coli K-12 fed to calves survived in the straw of the pen for an additional 3 days but could not colonize animals introduced into the contaminated environment. Hence, not only do E. coli K-12 cells at least survive in the alimentary tract and after voiding but they also can transmit and receive plasmids at a very low rate. It is also clear from our studies that a carried plasmid may have a profound effect on the survival and carriage of E. coli K-12. As noted earlier, many E. coli can be converted into a pathogenic form following the infection with Ent and K antigen plasmids. If E. coli K-12 is so infected with Ent + K99 the organism is not capable of inducing overt disease in calves. Nevertheless, while ordinary E. coli K-12 can only survive for 3 days in calves, K-12 cells carrying the Ent plasmid multiply in the calf gut and are excreted for at least 8 days, whereas K-12 cells harboring both Ent + K99 plasmids multiply and survived for 14 days (when the experiment was terminated). Of course, neither Ent nor K99 can be considered as typical in the sense that that they specifically evolved, at least in part, to confer selection advantage to host cells with regard to colonization of calves. Yet, it may not be too far fetched to suggest that some DNA recombinant molecules could profoundly affect the ability of this E. coli strain to survive and multiply in the gastrointestinal tract.

The results with E. coli K-12 are by no means unique to this strain alone since as noted earlier ingestion of cultures usually does not lead to permanent colonization. Neither Anderson, Smith (nor me) employed antibiotic selection. If this had been done the data would have been biased towards increased survival and higher incidences of plasmid transfer. Indeed, in the main one has consider-

able difficulty in detecting plasmid transfer *in vivo* without some form of selective pressure. In this context it is also interesting to note that Richmond fed 3 volunteers various *E. coli* containing R plasmids. In the absence of antibiotic selection these strains survived an average of 3 days. If the same individuals were fed the same strains coincident with a 5 day course of ampicillin therapy the strains survived an average of 50 days.

With regard to the interpretation of these findings as it affects our deliberation on DNA recombinant molecules I think several points need to be considered. The data on survival point out that ingested *E. coli* K-12 in numbers as small as 10^4 can survive in the bowel. One may see in comparing Smiths data with that of Anderson some degree of disagreement with regard to mean survival time. This, I believe is in part a reflection of the mode of ingestion. Smith directly ingested broth cultures whereas Anderson's subjects ingested their cultures in milk. I think the observed differences between the survival time seen by Smith (about 3 days) and those seen by Anderson (about 6 days) reflects the protection afforded the ingested organisms by the milk. Indeed, it is a well-established principle to use milk or $NaHCO_3$ along with dosing organisms when performing experiments on pathogenicity to increase the liklihood of infection. In a practical vein, this simply underscores the point that eating in the laboratory is a poor practice.

Smith, Anderson and most other workers who have studied *in vivo* plasmid transfer have not cited a precise frequency or probability of transmission *in vivo*. It is obviously very low and even if one employs an R plasmid known to have a probability of transfer of 1×10^{-1}/hour/ml of culture *in vitro*, *in vivo* transmission has never been observed (of which I am aware) that would exceed 1×10^{-10} per 24 hours/g of feces. It is important to keep in mind that, at present and I dare say in future, the plasmid species employed for cloning vehicles are non-selftransmissible i.e., they require some transmissible plasmid to mobilize them into a recipient cell. All of the work done by Smith,

Anderson, Richmond and others have employed transmissible plasmids. Mobilization of plasmids such as pSC101, RSF1010 and ColE1 from E. coli in vitro occur at a frequency of from a high of about 5×10^{-1} (for ColE1 by F) to a low of about 10^{-8} (for pSC101 by F). On average, however, mobilization occurs in vitro at a frequency of about $10^{-4} - 10^{-5}$/ml in 8 hours mixed cultivation. Thus, one would expect that the probability of an ingested E. coli K-12 strain transferring its recombinant DNA molecule in which ColE1 or pSC101 were used as cloning vehicle would probably not be greater than 1×10^{-12}/g feces in 24 hours.

I know of no comparable data that exists for phage transmission in vivo and presumably this is one area in which it would be useful to have contract studies performed. In terms of plasmid transmission in vivo, it is my personal opinion that sufficient evidence exists about the general phenomenon. However, when a suitable E. coli strain has been constructed that we feel might be recommended to workers in the field then it would be useful to determine its survival in vivo and the mobilization of the preferred cloning plasmid or cloning phage from this host.

Addendum:

The molecular definition of *Escherichia coli*:

The genus Escherichia and the 'species' *E. coli* was defined on biochemical and serological criteria. It is a reasonable view that variations exist among strains of *E. coli* but to what extent? We (Brenner et. al., J. Bacteriol. 109: 953, 1972) examined this question by asking in what sense do the populations of cells which we conveniently call *E. coli* diverge in overall genetic organization and in nucleotide sequence. Some 60 typical *E. coli* strains which included *E. coli* strains K12 and B as well as strains isolated from human, mammalian and vertebrate sources from varied geographical sources were examined. These strains varied by as much as 25% in their nucleotide sequences and their genome sizes varied from a low of 2.3×10^9 daltons to a high of 2.97×10^5 daltons. The frequency distribution of relatedness between *E. coli* K-12 and Shigella species overlaps the distribution seen among *E. coli* strains. However, no strains from any other genus of enteric bacteria show greater than 25% relatedness to *E. coli*. Thus, while there is an astronomical number of distinct cultures which can be independently isolated from animals which would be almost universally accepted by microbiologists as representing the species *Escherichia coli*, it is apparent that many display substantial differences in their overall organization and in their genetic fine structure. To what extent these differences may be correllated with ecological preferences and pathogenicity is not known.

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st or substrate	Number					Percent				
	+ 1-2*	(+) 3-7	(+) 8-14	(+) > 14	−	+ 1-2*	(+) 3-7	(+) 8-14	(+) > 14	−
drogen sulfide	0				1887	0				100
ase	0				1887	0				100
ol	1817				70	96.3				3.7
phyl red	1886				1	99.9				0.1
ges-Proskauer	0				1887	0				100
rate (Simmons')	4	6			1887	0.2	0.3			99.5
N (1224)**	32				1192	2.6				97.4
tility	1171				716	62.1				37.9
latin (22 C) (386)	0				386	0				100
sine decarboxylase (872)	703	13			156	80.6	1.5			17.9
ginine dihydrolase (872)	142	341			389	16.3	39.1			44.6
nithine decarboxylase (872)	498	70			304	57.1	8			34.8
enylalanine deaminase (1084)	0				1084	0				100
ose acid	1887				0	100				0
gas	1736				151	92				8
ctose	1729	64	13	2	79	91.6	3.4	0.7	0.1	4.2
erose	1013	87	13	4	770	53.7	4.6	0.7	0.2	40.8
annitol	1830				47	97.5				2.5
elcitol	930	317	23		617	49.3	16.8	1.2		32.7
licin	679	202	24	6	976	36	10.7	1.3	0.3	51.7
donitol	98	8			1781	5.2	0.4			94.4
ositol	17	4			1866	0.9	0.2			98.9

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TABLE 1 – Continued
THE BIOCHEMICAL REACTIONS OF *E. COLI*

Test or substrate	Number					Percent				
	+ 1-2*	(+) 3-7	(+) 8-14	(+) >14	–	+ 1-2*	(+) 3-7	(+) 8-14	(+) >14	–
Sorbitol	1515	19	2		351	80.3	1	0.1		18.6
Arabinose	1874	9			4	99.3	0.5			0.2
Raffinose	932	25	13	2	915	49.4	1.3	0.7	0.1	48.5
Rhamnose	1576	32	32		247	83.5	1.7	1.7		13.1
Malonate (419)	0				419	0				100
Mucate (344)	315				29	91.6				8.4
Christensen's citrate (469)	85	105	1		278	18.1	22.4	0.2		59.3
Jordan's tartrate (210)	205				1	97.6				2.4
Sodium pectate (156)	0				156	0				100
Sodium acetate (186)	156	18			12	83.8	9.7			6.5
Ammonium salts glucose agar (238)	225	4			9	94.5	1.7			3.8
Sodium alginate (156)	0				156	0				100
Linase, corn oil (156)	0				156	0				100
Maltose (773)	700	14	1	4	54	90.6	1.8	0.1	0.5	7
Xylose (517)	428	28	6		55	82.8	5.4	1.2		10.6
Trehalose (379)	372	7			0	98.2	1.8			0
Cellobiose (409)	15	13	6	6	369	3.7	3.2	1.5	1.4	90.2
Glycerol (373)	332	31			10	89	8.3			2.7
Alpha methyl glucoside (152)	0				152	0				100
Erythritol (152)	0				152	0				100
Esculin (152)	47	30			75	30.9	19.7			49.3
Nitrate to nitrite (412)	411				1	99.8				0.2
Oxidation-fermentation (400)	400F					100F				
Oxidase (409)	0				409	0				100
HCA (193)	13				180	6.7				93.3

*Days of incubation

**Number tested if different from total

TABLE 2

SUMMARY OF THE BIOCHEMICAL REACTIONS OF *E. COLI*

(Based upon data in Table 1)

Test or substrate	Sign	%+	(%+)
Hydrogen sulfide	–	0	
Urease	–	0	
Indol	+	96.3	
Methyl red	+	99.9	
Voges-Proskauer	–	0	
Citrate (Simmons')	–	0.2	(0.3)
KCN	–	2.6	
Motility	+ or –	62.1	
Gelatin (22 C)	–	0	
Lysine decarboxylase	d	80.6	(1.5)
Arginine dihydrolase	d	16.3	(39.1)
Ornithine decarboxylase	d	57.8	(8)
Phenylalanine deaminase	–	0	
Glucose acid	+	100	
gas	+	92	
Lactose	+	91.6	(4.2)
Sucrose	d	53.7	(5.5)
Mannitol	+	97.5	
Dulcitol	d	49.3	(18)
Salicin	d	36	(12.3)
Adonitol	–	5.2	(0.4)
Inositol	–	0.9	(0.2)
Sorbitol	d	80.3	(1)

See footnote and key at end of table.

TABLE 2 – Continued
SUMMARY OF THE BIOCHEMICAL REACTIONS OF *E. COLI*
(Based upon data in Table 1)

Test or substrate	Sign	%+	(%+)
Arabinose	+	99.3	(0.5)
Raffinose	d	49.4	(2.1)
Rhamnose	d	83.5	(3.4)
Malonate	–	0	
Mucate	+	91.6	
Christensen's citrate	d	18.1	(22.6)
Jordan's tartrate	+	97.6	
Sodium pectate	–	0	
Sodium acetate	+ or (+)	83.8	(9.7)
Ammonium salts glucose agar	+	94.5	(1.7)
Sodium alginate	–	0	
Lipase, corn oil	–	0	
Maltose	+	90.6	(2.4)
Xylose	d	82.8	(6.6)
Trehalose	+	98.2	(1.8)
Cellobiose	–	3.7	(6.1)
Glycerol	+ or (+)	89	(8.3)
Alpha methyl glucoside	–	0	
Erythritol	–	0	
Esculin	d	30.9	(19.7)
Nitrate to nitrite	+	99.8	(0.2)
Oxidation-fermentation	F	100	
Oxidase	–	0	
HCA	–	6.7	

*Figures in parentheses indicate percentages of delayed reactions (3 or more days).

Key to Symbols:
+ 90% positive within 1 or 2 days' incubation.
– no reaction (90% or more).
+ or – majority of strains positive, some cultures negative.
+ or (+) majority of reactions occur within 1 or 2 days, some are delayed.
d different reactions: +, (+), –.
F fermentative reaction.

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PUBLIC HEALTH SERVICE
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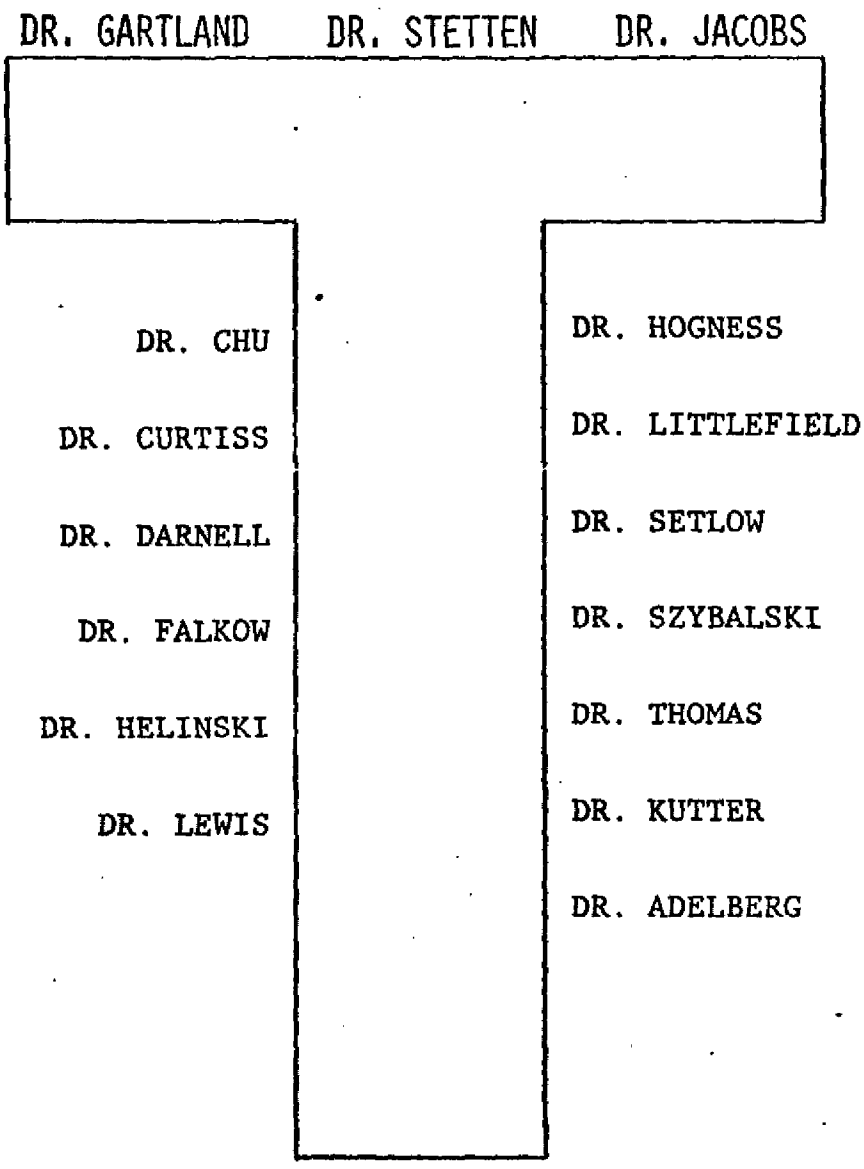

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